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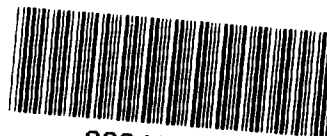
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August 24, 1994  
MRS-134-94

Hoechst Celanese Corporation  
Route 202-206  
PO Box 2500  
Somerville, NJ 08876-1258  
908 231 2000  
Telex 833 449  
Fax 908 231 4554

Attn: TSCA Section 8(e) Coordinator  
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Dear Sir or Madam:

In accordance with the requirements of TSCA Section 8(e), Hoechst Celanese hereby submits an In Vitro test for Chromosome Aberrations in Chinese Hamster V79 Cells for beta-hydroxynaphthoic acid (CAS No. 92-70-6) because the test material induced a significant increase in the number of chromosome aberrations.

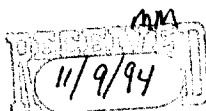
Three study reports were received from Hoechst AG:

- 1) Mutagenic Potential of the Compound in Strains of Salmonella typhimurium (Ames Test) and Escherichia coli (Report No. 370/82),
- 2) Chromosome Aberrations In Vitro in V79 Chinese Hamster Cells (Report No. 89.0025),
- 3) Chromosome Aberrations In Vivo Cytogenetic Test in Bone Marrow Cells of the Chinese Hamster (Report No. 93.0733).

These studies will henceforth be referred to as the Ames Test, In Vitro Cytogenetics, and In Vivo Cytogenetics, respectively.

The test material was not mutagenic in the Ames Test conducted using five strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538) and Escherichia coli WP2uvrA, with and without metabolic activation. Similar results are reported in the literature (Shimizu, H. et al., Sangyo Igaku 27(6):400-419, 1985).

In the In Vitro Cytogenetics assay, the test material induced a significant increase in the number of chromosome aberrations in chinese hamster V79 cells 18 hours after treatment with 750  $\mu$ g/ml in the absence of metabolic activation. This increase was substantially greater than the increase induced by the positive control material. The test material was not clastogenic (i.e. did not induce chromosome aberrations) in the presence of metabolic activation at 150  $\mu$ g/ml, the highest concentration tested with activation (due to cytotoxicity at higher levels).



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In addition, the test material was not clastogenic in the In Vivo Cytogenetics assay. No increase in chromosome aberrations was noted in bone marrow cells harvested from chinese hamsters sacrificed 12, 24, or 48 hours after a single oral dose of 2000 mg/kg bodyweight, a limit dose which showed no signs of clinical toxicity.

Thus, although BONS appears to be a potent clastogen in vitro without the benefit of metabolic activation, it produced no effect in the more relevant in vivo study, where absorption, distribution, metabolism, and excretion are involved.

Finally, beta-hydroxynaphthoic acid (92-70-6) is a TSCA Section 8(d) listed chemical. Hoechst Celanese is submitting under TSCA Section 8(d) the above mentioned studies as well as the attached summary information from our parent company, Hoechst AG.

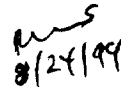
This submission contains no confidential business information.

If any further information is required, do not hesitate to contact Dr. Michele R. Sullivan, Director, Product Stewardship at 908-231-4480.

Sincerely,



Susan Engelman  
Vice President, Environmental, Health & Safety Affairs



Encl.

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Report No. 89.01  
January 18th, 1989  
Page 1 (28)

BON-S

Study Title

beta-Oxynaphthoesaeure

CHROMOSOME ABERRATIONS

IN VITRO

IN V79 CHINESE HAMSTER CELLS

Author

Dr. W. Müller

Study completed on

1989-01-12

Performing Laboratory

Pharma Research Toxicology and Pathology  
Hoechst Aktiengesellschaft  
Postfach 80 03 20  
6230 Frankfurt am Main 80

Laboratory Project ID

Study No. 88.1243

# Hoechs

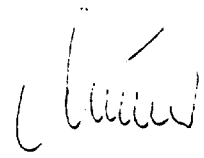
Report No. 89.0025  
January 18th, 1989  
Page 2 (28)

This report contains the unpublished results of research conducted by HOECHST AKTIENGESELLSCHAFT. These results must not be published, either wholly or in part, or reviewed or quoted in any other publication without the authorization of the company.


## STATEMENT OF COMPLIANCE

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director

Dr. Müller 

Head of Toxicology

Dr. Mayer 

Report No. : 89.0025  
Page 4 (28)

## Quality Assurance Statement

Hoechst Aktiengesellschaft  
Pharma Research  
Quality Assurance (GLP)

23.02.1989

Title : beta-Oxynaphthoesaeure  
CHROMOSOMEM ABERRATIONS  
IN VITRO  
IN V79 CHINESE HAMSTER CELLS

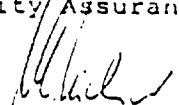
Date : 18.01.1989

Study No. : 88.1243

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below :

Inspection	Report
05.09.1988	05.09.1988
25.10.1988	25.10.1988
22.02.1989-23.02.1989	23.02.1989

Pharma Research  
Quality Assurance (GLP)



## CONTENTS

	PAGE
GLP - STATEMENT.....	4
1. SUMMARY.....	6
2. INTRODUCTION.....	7
3. GENERAL.....	8
4. MATERIALS AND METHODS.....	9 - 14
4.1 Test compound.....	9
4.2 Preparation and storage of a liver homogenate fraction (S9).....	10
4.3 Preparation of S9-mix.....	10
4.4 Mammalian cells.....	10
4.5 Experimental design.....	10
4.6 The controls.....	12
4.7 Experimental groups.....	12
4.8 Analysis of metaphases.....	13
4.9 Evaluation.....	14
5. RESULTS.....	15 - 16
5.1 Solubility and toxicity.....	15
5.2 Mutagenicity test.....	15 - 16
6. APPENDIX.....	17
6.1 Examples of aberrations.....	17
7. REFERENCES.....	18
8. TABLES.....	19 - 28

## 1. SUMMARY

The test substance beta-Oxynaphthoesaeure was examined for mutagenic activity in V79 Chinese hamster cells. The induction of chromosome aberrations after in vitro treatment was investigated in the presence and absence of a fraction of liver homogenate for metabolic activation (S9-mix).

A preliminary cytotoxicity experiment was performed in order to select appropriate dose levels for the mutagenicity study. The test substance produced a high cytotoxic effect (reduction of plating efficiency) without metabolic activation from 1000 ug/ml up to the limit of solubility (1890 ug/ml). Cytotoxic effects was also observed with metabolic activation from 200 ug/ml up to the limit of solubility. For mutagenicity testing two independent cell cultures with and without metabolic activation (S9-mix) were used.

For main experiment dose levels of 750, 250, 75 ug/ml in the absence of S9-mix and dose levels of 150, 75, 10 ug/ml in the presence of S9-mix were used.

The test compound beta-Oxynaphthoesaeure induced a significant increase in the number of chromosome aberrations 18 h after treatment with 750 ug/ml without S9-mix. A slight increase of aberrations was observed at the dose level of 750 ug/ml 6 h after treatment in the absence of S9-mix inclusive gaps.

In conclusion beta-Oxynaphthoesaeure induced chromosome mutations (=aberrations) in V79 Chinese hamster cells, in the absence of a metabolic activation system, under the experimental conditions described in this report.

## 2. Introduction

The in vitro cytogenetic test is a mutagenicity test system for the detection of chromosomal aberrations in cultured mammalian cells (1). This system offers several advantages. When modern and adequate techniques are employed cell cultures show only minor variations between a series of passages with respect to the cell cycle, viability, plating efficiency, medium requirements and karyotype. Furthermore the cells can be stored frozen as stock for many experiments and for reference.

Chromosomal aberrations may be either structural or numerical. However, because cytogenetic assays are usually designed to analyse cells at their first post-treatment mitosis and numerical aberrations require at least one cell division to be visualized, this type of aberration is generally not observed in a routine cytogenetic assay. Structural aberrations may be of two types: chromosome or chromatid aberrations.

Chromosome-type aberrations are induced when a compound acts in the G<sub>1</sub> phase of the cell cycle. Chromatid-type aberrations are induced when a chemical acts in the S or G<sub>2</sub> phase of the cell cycle.

- Chromosome-type aberrations are changes which result from damage expressed in both sister chromatids at the same locus
- Chromatid-type aberrations result from damage expressed as breakage of a single chromatid or breakage and/or reunion between chromatids
- Numerical aberrations are variations of the normal chromosome number characteristic of the cells used in the assay

The V79 cell line has been used successfully for many years in in vitro experiments. Especially the high proliferation rate (doubling time 12-16 hours in stock cultures) and a high plating efficiency of untreated cells, both necessary for the appropriate performance of the study, recommend the use of this cell line (2,3,4).

### 3. GENERAL

Study-No. : 88.1243  
Test compound : beta-Oxynaphthoesaeure  
Code : GSVB 155  
Ordered by : Werk Offenbach, Produktion  
Test system : in vitro mammalian cytogenetic test  
Test organism : cells of Chinese hamster cell line V79  
Initiation of the study : November 8th, 1988  
Termination of the study : November 10th, 1988

### R e s p o n s i b i l i t y

Head of Genetic Toxicology: Dr. MÜLLER

Head of Toxicology : Dr. MAYER

Quality assurance unit : Ap. HARSTON

Testing facilities and archives: Pharma Research Toxicology and Pathology  
HOECHST AKTIENGESELLSCHAFT  
P.O. box 80 03 20  
6230 Frankfurt 80

## 4. MATERIAL AND METHODS

### 4.1. Test compound

Name	: beta-Oxynaphthoesaeure
Code	: GSV8 155
Other names	: BONS
CAS No.	: 92-70-6
Chemical nomenclature	: 2-Naphthalenecarboxylic acid, 3-hydroxy
Molecular formula	: $C_{11}H_8O_3$
Purity	: 98.5 %
Impurity	: 1.0 % B-Naphtol
Appearance	: yellow powder
Melting point	: 218 °C
Molecular weight	: 188
Charge No.	: Pt.680/88
Date of submission	: September 6th, 1988
Storage conditions	: dark at 20 °C
Cell culture medium	: MEM (Minimal essential medium) with Hanks-salts and 25 mM Hepes-buffer

At the day of the experiment the test substance was dissolved as a solution in methanol at appropriate concentrations. Two independent cell cultures (No. 1 and 2) were used.

## 4.2 Preparation and storage of a liver homogenate fraction (S9)

Male Sprague Dawley rats (200-300 g) received a single intraperitoneal injection of Aroclor 1254 (500 mg/kg bodyweight) 5 days before sacrifice. Preparation is performed at 0 to 4 °C using cold sterile solution and glassware. The livers from at least 5-6 animals are removed and pooled, washed in 150 mM KCl (approximately 1 ml/g wet livers). The washed livers are cut into small pieces and homogenized in three volumes of KCl. The homogenate is centrifuged at 9000 g for 10 minutes. The supernatant is the S9 fraction. It is divided into small portions, rapidly frozen and stored at -80 °C for not longer than three months.

## 4.3 Preparation of S9-mix

Sufficient S9 fraction is thawed immediately before each test at room temperature. One volume of S9 fraction is mixed with 9 volumes of the S9 cofactor solution and kept on ice until its use. This preparation is termed S9-mix. The concentrations of the different compounds in the S9-mix are:

8 mM MgCl<sub>2</sub>  
33 mM KCl  
5 mM glucose-6-phosphate  
4 mM NADP<sup>+</sup>  
100 mM phosphate buffer pH 7.4

## 4.4 Mammalian cells

Large stocks of the V79 cell line stored in liquid nitrogen in the Laboratory of Genetic Toxicology of Hoechst AG are allowing the repeated use of the same cell culture batch in experiments. Consequently the cellular parameters of the experiments remain similar because of the reproducible characteristics of the cells.

The tawed stock cultures were propagated at 37 °C in 25 cm<sup>2</sup> plastic flasks. Seeding was done with about  $1-3 \times 10^5$  cells per flask in 5 ml of MEM-medium supplemented with 10 % fetal calf serum (FCS). The cells were subcultured twice a week.

## 4.5 Experimental design

Two days old exponentially growing stock cultures which were over 50 % confluent were trypsinised and a single cell suspension was prepared. The trypsin concentration was 0.5 % in Ca-Mg-free salt solution.  $1-2 \times 10^6$  cells/flask were seeded into four 80 cm<sup>2</sup> plastic flasks containing 15 ml MEM with 10 % FCS (6 h preparation).  $4-6 \times 10^5$  cells/flask were seeded into four 25 cm<sup>2</sup> plastic flasks containing 5 ml MEM with 10 % FCS (18 h preparation).  $2-4 \times 10^5$  cells/flask were seeded into four 25 cm<sup>2</sup> plastic flasks containing 5 ml MEM with 10 % FCS (28 h preparation).

After 24 h the medium was replaced with medium containing 5 % FCS and the test substance, both without S9-mix and with 40 ul/ml S9-mix.

After 2 h this medium was replaced with normal medium after rinsing once with physiological saline solution.

Treatment was performed with 3 concentrations of the test substance.

The highest concentration did not reduce the number of scorable metaphases more than 20 % of the negative controls. The mitotic index was determined in samples of 1000 cells. The toxicity of the test substance was determined in a preliminary experiment by establishing the concentration-related plating efficiency. According to these data the concentration range was chosen.

3.5, 15.5 and 25.5 h after the start of the treatment colcemide was added (0.04 ug/ml culture medium) to the cultures. 2.5 h later (6 h, 18 h and 28 h preparation) the cells were trypsinised.

For hypotonic treatment, approximately 5 ml of 0.075 M potassium chloride solution at 37 °C was quickly added and suspended. This suspension was then allowed to incubate for 10 minutes in a water bath at 37 °C. Addition of 1.5 ml fixative and flow through with air.

After re-centrifuging for five minutes at 1000 rpm, all but one drop of the supernatant was drawn off by pipette. The sediment was carefully covered with a layer composed of 2.5 ml fixative (methanol : glacial acetic acid 3 + 1). After 20 minutes the fixation was removed carefully with a pipette and suspended in 2.5 ml fixative. After another 30 minutes, the mixture was centrifuged, after which the liquid was removed by pipette and fresh fixative added. The tubes were covered and kept for at least 12 hours (overnight) in a refrigerator at 4 °C.

After re-centrifuging for 5 minutes at 1000 rpm, all but one drop of the liquid was removed by pipette and a new suspension formed with a small quantity of freshly prepared fixative. A few drops of this suspension were placed with a pasteur pipette onto clean microscopic slides which had been stored in distilled water at 4 °C, the drops were then briefly passed through a Bunsen flame and air-dried for 24 hours. Staining was performed as follows:

- staining for 10 minutes in 2 % orcein solution
- rinsing 3 times in distilled water
- rinsing twice in acetone
- brief rinsing in acetone/xylene
- 2 minutes in acetone/xylene
- 5 minutes in xylene
- 10 minutes in xylene
- embedding in Entellan<sup>R</sup> or Eukitt<sup>R</sup>

2-5 slides were prepared from each flask.

In the same way both negative and positive controls were prepared 16 h after medium change or treatment, respectively.

## 4.6 The controls

### Negative controls:

Untreated cultures and cultures treated with the solvent.

### Positive controls:

Without metabolic activation:

EMS (Ethylmethanesulfonate) dissolved in nutrient medium in a concentration of 2000 ug/ml. The solution was prepared on the day of the experiment.

With metabolic activation:

CPA (Cyclophosphamide) - Endoxan final concentration in nutrient medium was 5 ug/ml. The solution was prepared on the day of the experiment.

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is proofed of the biological stability.

## 4.7 Experimental groups

### Preparation time 18 h

Negative control:	untreated cells	
Negative control:	untreated cells + S9-mix	
Solvent control :	cells + solvent	
Solvent control :	cells + solvent + S9-mix	
Positive control:	cells + EMS	
Positive control:	cells treated with CPA + S9-mix	
Test group 1:	cells + test substance	75 ug/ml
Test group 1:	cells + test substance + S9-mix	10 ug/ml
Test group 2:	cells + test substance	250 ug/ml
Test group 2:	cells + test substance + S9-mix	75 ug/ml
Test group 3:	cells + test substance	750 ug/ml
Test group 3:	cells + test substance + S9-mix	150 ug/ml

### Preparation time 6 and 28 h

Solvent control :	cells + solvent	
Solvent control :	cells + solvent + S9-mix	
Test group 3:	cells + test substance	750 ug/ml
Test group 3:	cells + test substance + S9-mix	150 ug/ml

The concentrations were chosen from the data of the cytotoxicity assay in the preliminary experiment as follows:

The survival rate of cells treated with the test substance.

<u>ug/ml</u>	<u>without S9-mix</u>	<u>with S9-mix</u>
Negative control	69.7 %	64.2 %
Solvent control	55.3 % = 100 %	59.8 % = 100 %
50	97.3 %	75.5 %
100	98.6 %	15.3 %
200	104.5 %	0.0 %
300	63.9 %	0.0 %
500	27.2 %	0.0 %
750	8.2 %	0.4 %
1000	0.0 %	2.1 %
1500	0.0 %	0.0 %
1880	0.0 %	0.0 %

#### 4.8 Analysis of metaphases

After the slides had been coded (Coding Scheme 1186/88), 100 metaphases per experimental group were examined. The set of chromosomes was examined for completeness and the various chromosomal aberrations were assessed. The chromosomal aberrations were classified as shown in chapter 6.1. Only metaphases with 22 - 1 chromosomes are included in the analysis. The metaphases were examined for the following aberrations: gap (g), break (b), fragment (f), minute (m), deletion (d), exchanges including intrachanges (ex), dicentrics (di), chromosome disintegration (cd) and ring formation (ri). In addition, metaphases with 5 and more aberrations were classified separately as multiple aberrations (ma).

After the metaphases had been evaluated, the code was lifted. The values for the control group were compared with the results from the dose group and the positive control at each preparation time.



## 4.9 Evaluation

The evaluation of the results was performed as follows:

- The test substance is classified as mutagenic if it induces a significantly increased aberration rate as compared with the negative controls with one of the concentrations tested. The significance is obvious either by an enhancement of the rate clearly exceeding the control range or it is proven by adequate biometry (Binomial statistic with Fisher's exact test).
- the test substance is classified as mutagenic if there is a reproducible concentration related increase in the aberration rate.
- the test substance is classified as not mutagenic when it tests negatively both with and without metabolic activation.

## 5. RESULTS

### 5.1 Solubility and toxicity

In a preliminary experiment beta-Oxynaphthoesaeure was assayed with respect to its solubility in cell culture medium. The highest concentration at which no visible precipitation was observed, was found to be 1880 ug/ml.

The cytotoxicity experiment proved that beta-Oxynaphthoesaeure was very toxic to the V79 cells in the absence of metabolic activation (S9-mix) from 1000 ug/ml and in the presence of S9-mix from 200 ug/ml up to the limit of solubility (1880 ug/ml) (page 13).

On the basis of these results the preparation of chromosomes was done after 2 h-treatment. In the presence of metabolic activation concentrations of 150 ug/ml at 6, 18 and 28 h, and additionally with 75 and 10 ug/ml at 18 h after treatment were prepared. Without S9-mix concentrations of 750 ug/ml at 6, 18 and 28 h and 250 and 75 ug/ml at 18 h after treatment were used.

### 5.2 Mutagenicity

The test substance beta-Oxynaphthoesaeure was assessed for its mutagenic potential in vitro in the chromosome-aberration-test with two independent cell cultures with and without metabolic activation (S9-mix). The results of these experiments are presented on tables 2 - 7. No significant toxic effect as reduction of the mitotic index was observed at any of the dose levels tested. The results are shown on table 1.

There was an enhancement of the aberration rates 18 h after the start of the treatment with 750 ug/ml without S9-mix. These data were found significantly enhanced in the Fisher's exact-test. The types of aberrations induced preliminary consisted of breaks, fragments, deletions, exchanges and minutes. This is an indication of heavy chromosomal damage.

Also at the preparation time of 6 h without S9-mix at the same concentration the aberration rates were enhanced, inclusive gaps.

Hoechst 

Report No. 89.0025  
January 18th, 1989  
Page 16 (28)

The results lead to the conclusion that beta-Oxynaphtoesaeure is mutagenic in the chromosome aberration test system in vitro with cells of the V79 Chinese hamster cell line in the absence of S9-mix under the conditions described in this report.

The sensitivity of the test system was demonstrated by the enhanced mutation frequency in the cell cultures treated with the positive control substances.


Dr. WM/MF

Quality assurance unit


3,

Pharma Research Toxicology and Pathology

HOECHST AKTIENGESELLSCHAFT

  
Dr. Müller  
Study Director

16/6/79

  
Dr. Mayer  
Head of Toxicology

## 6. APPENDIX

### 6.1 Examples of aberrations

#### 1. Structural aberrations

Gap: Non-stained segment (achromatic gap) of chromatide without dislocation of the apparently separate part, irrespective of size of the non-stained area.

Break: A visible fracture of the chromatide structure where the broken piece is laterally dislocated or shifted in the longitudinal axis but can still be assigned to the corresponding centric part.

Fragment: Acentric part of a chromosome which may appear individually, regardless of its size.

Minute: Small chromatide body with a diameter smaller than the width of the chromatide.

Deletion: Terminal or interstitial losses of part of the chromatide.

Exchange: These are exchange aberrations, subdivided into intrachanges (the union of parts that can combine, within a chromosome) and interchanges (the union of parts that can combine from two or more chromosomes). Dicentric chromosomes and ring chromosomes are included in this group.

The chromatide aberrations specified above can also occur as iso-chromatide aberrations (e.g. isochromatide break)

#### 2. Numerical aberrations

Aneuploidy: A deviation from the typical number of individual chromosomes in a set of chromosomes; a decrease in the number is known as hypoploidy and an increase as hyperploidy.

Polyploidy: More than two sets of chromosomes.

#### 3. Additional criterion:

Chromosomal disintegration: Where all or most of the chromosomes are irregular particles. If exchange figures occur in the metaphases, they are only included in this aberration group.

## 7. REFERENCES

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- 6) EPA In Vitro Mammalian Cytogenetics  
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Office of Toxic Substances  
Office of Pesticides and Toxic Substances  
U.S. Environmental Protection Agency  
Washington, D.C. 20460

## 8. TABLES

Table 1: Mitotic index

Test group	Dose ug/ml	S9 mix	fixation interval (h)	mitotic index per cent*		abs.	rel.
				slide 1	slide 2		
Solvent control	0	-	6	9.7	9.2	9.5	100.0
Test article	750	-	6	8.0	6.1	7.1	74.7
Solvent control	0	+	6	12.6	12.5	12.6	100.0
Test article	150	+	6	7.4	6.3	6.9	54.8
Negative control	0	-	18	12.5	8.6	10.6	100.0
Solvent control	0	-	18	11.0	9.4	10.2	100.0
Positive control EMS	2000	-	18	11.4	11.2	11.3	110.8
Test article	75	-	18	11.2	8.9	10.1	99.0
Test article	250	-	18	11.0	9.5	10.3	101.0
Test article	750	-	18	12.7	12.2	12.5	122.5
Negative control	0	+	18	16.0	8.8	12.4	100.0
Solvent control	0	+	18	14.7	10.1	12.4	100.0
Positive control CPA	5	+	18	11.7	11.6	11.7	94.4
Test article	10	+	18	14.3	12.2	13.3	107.3
Test article	75	+	18	9.6	9.0	9.3	75.0
Test article	150	+	18	10.5	9.2	9.9	79.8
Solvent control	0	-	28	15.5	14.7	15.1	100.0
Test article	750	-	28	12.4	11.2	11.8	78.1
Solvent control	0	+	28	16.4	14.6	15.5	100.0
Test article	150	+	28	10.8	14.0	12.4	80.0

\* The mitotic index was determined in 1000 cells from each of two slides per test group

Table 2: Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 6 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture without S9 mix	No. of phases with aberrations incl. Gaps	No. of aberrations incl. Gaps	g	ig	b	tb	f	if	d	id	ma	ex	cd	others	MI %
0	S/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.7
0	S/2	1	0	1	0	1	0	1	0	1	0	0	0	0	0	9.2
Total		1	0	1	0	1	0	0	0	0	0	0	0	0	0	
750	3/1	3	0	3	0	3	0	3	0	3	0	0	0	0	0	8.0
750	3/2	6	3	6	3	6	3	3	1	2	0	0	0	0	0	6.1
Total		9*	3	9*	3	9*	3	6	1	2	0	0	0	0	0	

S = solvent control

\* =  $p < 0.05$

Table 3: Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 6 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture with S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI *
0	S/1	1	0	0	1	0	0	1								12.6
0	S/2	1	0	0	1	0	0	1								12.5
Total		2	0	0	2	0	0	2								
150	3/1	4	0	0	4	0	0	4								7.4
150	3/2	1	0	0	1	0	0	1								6.3
Total		5	0	0	5	0	0	5								

S = solvent control

Table 4 : Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 18 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture without S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
0	N/1	1	0	1	0	1										12.5
0	N/2	1	0	1	0	1										8.6
Total		2	0	2	0	2										
0	S/1	2	1	2	1	1									rf	11.0
0	S/2	0	0	0	0	0										9.4
Total		2	1	2	1	1									1	
2000	P/1	10	8	11	9	2				3			6			11.4
2000	P/2	11	10	11	10	1	1			1			7		df	11.2
Total		21*	18*	22*	19*	3	1			4			13		1	

N = negative control    S = solvent control    P = positive control    \* = p < 0.05

Table 4 (cont.): Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 18 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture without S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
75	1/1	2	2	2	2											m,ri 11.2
75	1/2	2	1	2	1	1										m 8.9
Total		4	3	4	3	1									3	
250	2/1	2	0	2	2	0	2									11.0
250	2/2	1	0	1	0	1										9.5
Total		3	0	3	0	3										
750	3/1	23	23	45	39	6	5	1	7	2	1	2	20		m	12.7
750	3/2	18	18	38	34	4	10	2	1	1			16		4m	12.2
Total		41*	41*	83*	73*	10	15	3	8	2	2	2	36		5	

\* =  $p > 0.05$

Table 5 : Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 18 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture with S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
0	H/1	0	0	0	0											16.0
0	H/2	0	0	0	0											8.8
Total		0	0	0	0											
0	S/1	1	0	0	1	0	1									14.7
0	S/2	1	0	0	1	0	1									10.1
Total		2	0	0	2	0	2									
5	P/1	8	7	11	8	3	1	2							2di,3m	11.7
5	P/2	6	6	8	8		2						2		3m,di	11.6
Total		14*	13*	19*	16*	3	1	2	2				2		9	

H = negative control    S = solvent control    P = positive control    \* = p < 0.05

Table 5 (cont.): Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 18 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture with S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
10	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.3
10	1/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.2
Total		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
75	2/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.6
75	2/2	1	0	0	1	0	0	1	0	0	0	0	0	0	0	9.0
Total		1	0	0	1	0	0	1	0	0	0	0	0	0	0	
150	3/1	2	1	1	2	1	1	1	0	0	0	0	0	0	0	10.5
150	3/2	2	2	2	3	3	3	0	0	0	0	0	3	0	0	9.2
Total		4	3	3	5	4	4	1	0	0	0	0	3	0	0	

Table 6: Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaeure  
Preparation: 28 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture without S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
0	S/1	1	0	1	1	0	1									15.5
0	S/2	2	1	2	1	1	1								m	14.7
Total		3	1	3	1	2									1	
750	3/1	2	1	2	1	1	1								m	12.4
750	3/2	4	4	5	5								3		2m	11.2
Total		6	5	7	6	1							3		3	

S = solvent control

**Table 7:** Chromosome aberrations in V79 cells  
Test substance: beta-Oxynaphthoesaure  
Preparation: 28 h after administration (100 metaphases were analysed)

Dose ug/ml	Culture with S9 mix	No. of phases with aberrations incl. excl. Gaps	No. of aberrations incl. excl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
0	S/1	0	0	0	0											16.4
0	S/2	1	0	1	0											14.6
Total		1	0	1	0											
150	3/1	2	1	2	1										1	10.8
150	3/2	0	0	0	0											14.0
Total		2	1	2	1										1	

S = solvent control

Table 8: Summary of results

Test group	Number of cells analysed	Dose ug/ml	S9 mix	fixation interval (h)	per cent incl. gaps	aberrant cells excl. gaps	exchanges
Solvent control	200	0	-	6	0.5	0.0	0.0
Test article	200	750	-	6	4.5	1.5	0.0
Solvent control	200	0	+	6	1.0	0.0	0.0
Test article	200	150	+	6	2.5	0.0	0.0
Negative control	200	0	-	18	1.0	0.0	0.0
Solvent control	200	0	-	18	1.0	0.5	0.5
Positive control EMS	200	2000	-	18	10.5	9.0	7.0
Test article	200	75	-	18	2.0	1.5	0.5
Test article	200	250	-	18	1.5	0.0	0.0
Test article	200	750	-	18	20.5	20.5	18.0
Negative control	200	0	+	18	0.0	0.0	0.0
Solvent control	200	0	+	18	1.0	0.0	0.0
Positive control CPA	200	5	+	18	7.0	6.5	2.5
Test article	200	10	+	18	0.0	0.0	0.0
Test article	200	75	+	18	0.5	0.0	0.0
Test article	200	150	+	18	2.0	1.5	1.0
Solvent control	200	0	-	28	1.5	0.5	0.0
Test article	200	750	-	28	3.0	2.5	1.5
Solvent control	200	0	+	28	0.5	0.0	0.0
Test article	200	150	+	28	1.0	0.5	0.0



Study Title

Beta-Oxynaphthoesäure (BONS)

CHROMOSOME ABERRATIONS

IN VIVO CYTOGENETIC TEST

IN BONE MARROW CELLS OF THE CHINESE HAMSTER

Author

Dr. I. Stammberger

Report completion date  
October 19th, 1993

Performing laboratory

Hoechst Aktiengesellschaft  
Pharma Development Central Toxicology  
D-65926 Frankfurt am Main

Laboratory Project ID: Study No. 93.0077



Pharma Development Central Toxicology

Report No. 93.0733  
Page 2 (25)

This report contains the unpublished research findings of Hoechst scientists. It should not be published, in whole or in part, or referred to in any publication without authorization from the company.



## STATEMENT OF COMPLIANCE

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director

I. Stammberger 19 Oct. '93  
Dr. I. Stammberger

Testing facility management:

W. D. Mayer 21 Oct. 1993  
Dr. W. D. Mayer

Quality Assurance Statement

Title:     Beta-Oxynaphthoelsäure (BONS)  
          CHROMOSOME ABERRATIONS  
          IN VIVO CYTOGENETIC TEST  
          IN BONE MARROW CELLS OF THE CHINESE HAMSTER

Study:     93.0077

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below:

<u>Inspection</u>	<u>Report</u>
10.05.1993	10.05.1993
11.05.1993	11.05.1993
07.09.1993	07.09.1993
18.10.1993	18.10.1993
18.10.1993	18.10.1993

Quality Assurance (GLP)

## CONTENTS

	PAGE
Quality Assurance - Statement.....	4
1. SUMMARY.....	6
2. INTRODUCTION.....	7
3. SYNOPSIS.....	8
4. MATERIAL AND METHODS.....	9 - 13
4.1 Test compound.....	9
4.2 Test species and animal husbandry.....	10
4.3 Test groups.....	11
4.4 Procedure of the assay.....	12
4.5 Preparation and staining of bone marrow.....	12
4.6 Analysis of metaphases.....	13
4.7 Evaluation.....	13
4.8 Biometry.....	13
5. RESULTS.....	14 - 15
5.1 Behaviour and mortality.....	14
5.2 Toxicity and mutation results.....	14 - 15
6. APPENDIX.....	16 - 25
6.1 Examples of chromosomal aberrations.....	16
6.2 Preliminary study.....	17
6.3 Tables of results.....	18 - 25



## 1. SUMMARY

**Beta-Oxynaphthoesäure (BONS)** was suspended in starch mucilage and dosed once orally at 2000 mg/kg bodyweight to male and female Chinese hamsters, based upon the results of the previously conducted dose range finding assay (see page 17).

According to the test procedure 5 males and 5 females from each group were killed 12, 24 or 48 hours after treatment by carbon dioxide asphyxiation.

Endoxan® used as positive control substance was administered orally at a dose of 50 mg per kg bodyweight.

The bone marrow obtained from femora of the animals was prepared, placed on microscopic slides and stained. 50 metaphases per animal were evaluated. The completeness in the number of chromosomes and the various chromatid and chromosomal aberrations were assessed.

In conclusion, **Beta-Oxynaphthoesäure (BONS)** did not induce a significant increase in the number of phases with aberrations in bone marrow cells of treated animals under the experimental conditions described in this report.

Marked increases of the chromosome aberrations were obtained with the positive control substance in males and females indicating the sensitivity of the assay.

The results indicate, **Beta-Oxynaphthoesäure (BONS)** is not mutagenic in the in vivo chromosome aberration test using bone marrow cells of the Chinese hamster.

## 2. INTRODUCTION

The purpose of this study was to establish the presence of a mutagenic risk resulting from exposure to the test substance. Cytogenetic investigations enable various types of chromosomal aberrations to be assessed. The Chinese hamster has proved to be a suitable test species and is recommended in various test guidelines.

The present study was conducted in accordance with the

EEC Directive 92/69, L 383 A, part B.11, p. 151 - 153

OECD Guideline for Testing of Chemicals, 475, April 1984  
"Genetic Toxicology - in vivo Mammalian Bone Marrow  
Cytogenetic Test - Chromosomal analysis"



### 3. SYNOPSIS

Study-No. : 93.0077  
Test compound : **Beta-Oxynaphthoesäure (BONS)**  
Code : HOE CG 0441 OD ZD98 0001  
Sponsor : GB: D, Werk Hoechst, UWS; Dr. Kern  
Test system : cytogenetic test in bone marrow cells  
Test species : Chinese hamster  
Route : oral  
Vehicle : starch mucilage  
Initiation of the study : May 10th, 1993  
Termination of the study : September 14th, 1993  
Dose levels : 0 and 2000 mg/kg bodyweight  
Positive control : Endoxan® 50 mg/kg bodyweight (oral)  
Number of animals : 5 males and 5 females from each dose group  
Killing times : 12, 24 or 48 hours after treatment  
(negative control and test compound)  
24h after treatment (positive control)

### R e s p o n s i b i l i t y

Head of Genetic Toxicology : Dr. I. STAMMBERGER  
Head of Toxicology : Dr. D. MAYER  
Quality assurance unit : Ap. S. HARSTON

Testing facilities and archives : Hoechst Aktiengesellschaft  
Pharma Development Central Toxicology  
D-65926 Frankfurt am Main



## 4. MATERIAL AND METHODS

### 4.1. Test compound

Name	: Beta-Oxynaphthoesäure (BONS)
Code	: HOE CG 0441 OD ZD98 0001
Synonyms	: 2-hydroxy-3-naphthoesäure, 2-hydroxynaphthalin-3-carbonsäure, C.I. Developer 20, Developer BON, Naphthol B.O.N.
CAS No.	: 000092-70-6
Charge No.	: 317/92 from July 16th, 1992
Certificate of analysis	: 04412 from October 01st, 1992, Analytical Laboratory Dr. Fischbach
Assay	: 97.9 %
Stability	: stable until July 1997 at room-temperature
Stability and homogeneity in vehicle	: guaranteed for 4 hours, proved in Analytical Laboratory Dr. Pletsch, dated May 05th, 1993
Chemical nomenclature	: 2-naphthalincarbonsäure,3-hydroxy-
Molecular formula	: $C_{11}H_8O_3$
Appearance	: light yellow, slight crystals
Melting point	: 220 °C
Molecular weight	: 188.18
pH - value in water	: 3.4
Date of delivery	: February 11th, 1993
Storage conditions	: dark at approximately 20 °C
Form of administration	: suspension
Positive control	: Cyclophosphamide - Endoxan <sup>®</sup> (Batch No. 091520)

## 4.2 Test species and animal husbandry

Test species	: Chinese hamster
Strain	: Han: Chin
Origin	: Zentralinstitut für Versuchstiere, Hannover
Age of animals	: 10 - 13 weeks
Number of animals	: 70
Bodyweight at start of study	: males : $\bar{x}$ = 31.8 g (27 - 37 g) females: $\bar{x}$ = 26.0 g (21 - 31 g)
Animal housing	: in fully air-conditioned rooms in Makrolon cages (Type 2) on soft wood granulate, one animal per cage
Room temperature	: 20 +/- 3 °C
Rel. atmospheric humidity	: approx. 30 - 70%
Lighting time	: 12 hours daily
Acclimatisation	: at least 5 days
Diet	: Altromin 7010 hamster diet (Altromin GmbH, Lage/lippe), <u>ad libitum</u>
Water	: tap water in plastic bottles, <u>ad libitum</u>
Animal identification	: cage numbering
Randomisation	: randomisation schedule 93.260 and 93.261

## 4.3 Test groups

The dose level of 2000 mg Beta-Oxynaphthoesäure (BONS) /kg bodyweight caused no signs of toxicity in the preliminary study (see page 17). Therefore it is used as the limit dose in the main study.

Group	Dose mg/kg bwt.	Conc. (%) (w/v)	Volume ml/kg bwt.	Number of animals per sex	Cage No. Animal No.	Killing (h after treatment)
1	0	0	10	5 males 5 females	1 - 5 6 - 10	12
2	2000	20.0	10	5 males 5 females	11 - 15 16 - 20	12
3	0	0	10	5 males 5 females	21 - 25 26 - 30	24
4	2000	20.0	10	5 males 5 females	31 - 35 36 - 40	24
5*	50	0.5	10	5 males 5 females	41 - 45 46 - 50	24
6	0	0	10	5 males 5 females	51 - 55 56 - 60	48
7	2000	20.0	10	5 males 5 females	61 - 65 66 - 70	48

\* Endoxan® (positive control, oral)

#### 4.4 Procedure of the assay

The test substance was administered orally to the test animals in a dose of 2000 mg/kg bodyweight. Starch mucilage was administered in the same way to the negative control groups. Simultaneously to negative controls and dose groups Endoxan<sup>R</sup> was used as positive control substance and was administered orally at a dose of 50 mg per kg bodyweight. Two hours before killing by carbon dioxide asphyxiation, each of the hamsters received an intraperitoneal injection of 3.3 mg demecolcin (Colcemid<sup>R</sup>) per kg bodyweight.

#### 4.5 Preparation and staining

After killing, both femora were removed and the bones completely stripped of muscle tissue. After removal of the epiphyses, the bone marrow was flushed out of the diaphysis (if necessary in alternate directions) into a centrifuge tube by means of a syringe containing Hanks solution (approx. 2 ml/femora) at a temperature of approx. 37 °C. This suspension was mixed and centrifuged for five minutes at approx. 1000 rpm. All but one drop of the supernatant was drawn off by pipette.

For hypotonic treatment, approximately 5 ml of approx. 0.075 M potassium chloride solution at approx. 37 °C was quickly added and suspended. This suspension was then allowed to incubate for 10 minutes in a water bath at approx. 37 °C. Approximately 1.5 ml fixative (methanol : glacial acetic acid 3 + 1) was then added and the suspension was bubbly mixed with air.

After re-centrifuging for approx. five minutes at approx. 1000 rpm, all but one drop of the supernatant was drawn off by pipette. The sediment was carefully covered with a layer composed of approx. 2.5 ml fixative. After at least 20 minutes, the fixation was carefully removed (after re-centrifuging) with a pipette and suspended in approx. 2.5 ml fresh fixative. In case of need the mixture was then centrifuged after another approx. 20 minutes, after which the liquid was removed by pipette and fresh fixative added. The tubes were covered and kept for at least 12 hours (overnight) in a refrigerator at approx. 4 °C.

After re-centrifuging for approx. five minutes at approx. 1000 rpm, all but one drop of the liquid was removed by pipette and a new suspension was formed with a small quantity of freshly prepared fixative. A few drops of this suspension were placed with a pasteur pipette onto clean microscopic slides. If necessary the slides were then briefly passed through a Bunsen flame and air-dried for at least 24 hours. Staining was performed as follows:

- staining for 10 minutes in approx. 2 % orcein solution
- rinsing 3 times in distilled water
- rinsing twice in acetone
- brief rinsing in acetone/xylene
- 2 minutes in acetone/xylene
- 5 minutes in xylene
- minimum 10 minutes in xylene
- embedding in Entellan<sup>R</sup> or Eukitt<sup>R</sup>

2-4 slides were prepared from each animal.

#### 4.6 Analysis of metaphases

After the slides had been coded (Coding Scheme 93.352 and 93.355), 50 metaphases per animal were examined. The set of chromosomes was examined for completeness and the various chromosomal aberrations were assessed. The chromosomal aberrations were classified as shown in the appendix 6.1. Only metaphases with 22 chromosomes are included in the analysis. The metaphases were examined for the following aberrations: gap (g), iso-gap (ig), break (b), iso-break (ib), fragment (f), iso-fragment (if), minute (m), iso-minute (im), deletion (d), iso-deletion (id), exchanges including intrachanges (ex), dicentrics (di), chromosome disintegration (cd), ring formation (ri) and polyploidy (pp). In addition, metaphases with 5 and more aberrations were classified separately as multiple aberrations (ma).

After the metaphases had been evaluated, the code was lifted. The values from control groups were compared with the results from the dose groups and the positive control.

#### 4.7 Evaluation

The evaluation of the results was performed as follows:

- the test substance is classified as mutagenic if it induces a statistically significant increased aberration rate (excluding gaps) as compared with the negative controls for at least one of the time points.
- the test substance producing no significant increase of the aberration rate is classified as non mutagenic.

#### 4.8 Biometry

Not necessary to perform as all mean chromosome aberration rates after treatment with the test article were in the range of the negative control values.

## 5. RESULTS

### 5.1 Behaviour and mortality

Animals were treated with 2000 mg Beta-Oxynaphthoesäure (BONS) per kg bodyweight to study various chromatid and chromosomal aberrations in bone marrow cells of the Chinese hamster.

All animals survived after application of 2000 mg Beta-Oxynaphthoesäure (BONS) per kg bodyweight. No signs of toxicity were observed.

The dissection of the animals revealed no test substance related macroscopic findings.

### 5.2 Toxicity and mutation results

Animals from each group were killed 12, 24 or 48 hours after treatment and examined for chromosomal aberrations in bone marrow cells. 5 males and 5 females were examined at each killing time. 50 metaphases per animal were evaluated. The sets of chromosomes were examined for completeness, and the incidence of various chromatid and chromosomal aberrations were recorded.

The findings for each animal are given in tables 2-8, arranged by test group, sex and killing time.

In addition, the findings were summarized in tabular form (table 1) and listed separately under the headings "inclusive gaps" and "exclusive gaps".

After administration of 2000 mg **Beta-Oxynaphthoesäure (BONS)** per kg bodyweight no indication of cytotoxicity as reduction of the mitotic index and no significant increase in the aberration rate exclusive and inclusive gaps was observed in the 12, 24 and 48 hour groups as compared with the negative controls. No increase of chromosome aberrations after administration of the test substance in all dose groups were observed.

The positive control substance **Endoxan<sup>®</sup>** was administered in a dose of 50 mg/kg bodyweight and produced a marked increase in the aberration rate among the animals killed after 24 hours as compared with the values for the negative controls. **Endoxan<sup>®</sup>** caused aberrations inclusive gaps in 14.0 % and exclusive gaps in 13.0 % of the metaphases. The positive control substance shows various types of aberrations and several aberrations per metaphase. So the sensitivity of the test system was demonstrated by the enhanced mutation frequency.

Under the experimental conditions described, it can be stated that the administration of **Beta-Oxynaphthoesäure (BONS)** did not lead to an increase of chromosome aberrations.

The results lead to the conclusion, **Beta-Oxynaphthoesäure (BONS)** is not mutagenic in the in vivo cytogenetic test using bone marrow cells of Chinese hamster.

Dr. IST/Ka

Quality Assurance (GLP)

3, 18.10.93

Pharma Development Central Toxicology

HOECHST AKTIENGESELLSCHAFT

*I. Stammberger* 19 Oct. 1993  
Dr. I. Stammberger  
Study Director

*D. Mayer* 21 Oct. 1993  
Dr. D. Mayer  
Head of Toxicology

## 6. APPENDIX

### 6.1 Examples of aberrations

#### 1. Structural aberrations

Gap: Non stained segment (achromatic gap) of chromatid without dislocation of the apparently separate part, irrespective of size of the non-stained area.

Break: A visible fracture of the chromatid structure where the broken piece is laterally dislocated or shifted in the longitudinal axis but can still be assigned to the corresponding centric part.

Fragment: Acentric part of a chromosome which may appear individually, regardless of their size.

Minute: Small chromatid body with a diameter smaller than the width of the chromatide.

Deletion: Terminal or interstitial losses of part of the chromatid.

Exchange: These are exchange aberrations, subdivided into intrachanges (the union of parts that can combine within a chromosome) and interchanges (the union of parts that can combine from two or more chromosomes). Dicentric chromosomes and ring chromosomes are included in this group.

The chromatide aberrations specified above can also occur as iso-chromatid aberrations (e.g. isochromatid break)

#### 2. Numerical aberrations

Aneuploidy: A deviation from the typical number of individual chromosomes in a set of chromosomes; a decrease in the number is known as hypoploidy and an increase as hyperploidy.

Polyploidy: More than two sets of chromosomes.

#### 3. Additional criterion:

Chromosomal disintegration: Where all or most of the chromosomes are irregular particles. If exchange figures occur in the metaphases, they are only included in this aberration group.

## 6.2 Preliminary study

Preliminary studies were conducted to determine the highest applicable non lethal dose level.

<u>1st dose</u>	:	800 mg/kg bodyweight <b>Beta-Oxynaphthoesäure (BONS)</b>
Observation period	:	April 28th - May 10th, 1993
Number of animals used	:	3 males and 3 females
Clinical signs	:	without clinical signs of toxicity
Lethality rate	:	0 out of 3 males 0 out of 3 females
 <u>2nd dose</u>	:	 1600 mg/kg bodyweight <b>Beta-Oxynaphthoesäure (BONS)</b>
Observation period	:	May 03rd - May 10th, 1993
Number of animals used	:	3 males and 3 females
Clinical signs	:	without clinical signs of toxicity
Lethality rate	:	0 out of 3 males 0 out of 3 females
 <u>3rd dose</u>	:	 2000 mg/kg bodyweight <b>Beta-Oxynaphthoesäure (BONS)</b>
Observation period	:	May 04th - May 10th, 1993
Number of animals used	:	3 males and 3 females
Clinical signs	:	without clinical signs of toxicity
Lethality rate	:	0 out of 3 males 0 out of 3 females

**Table 1:** **Summary:** Percentage of metaphases with aberrations per trial group (10 animals per group; 50 metaphases per animal)

Trial group	Dose mg/kg bodyweight	Killing time hours after admin.	Metaphases with aberrations		Metaphases with aberrations		Metaphases with exchanges
			inclusive gaps	SD	exclusive gaps	SD	
negative control Beta-Oxynaphthoe- säure (BONS)	0	12	0.4	0.84	0.0	0.00	0.0
	2000	12	1.6	2.06	0.0	0.00	0.0
negative control Beta-Oxynaphthoe- säure (BONS) Endoxan <sup>a</sup>	0	24	0.8	1.40	0.2	0.64	0.0
	2000	24	0.8	1.04	0.0	0.00	0.0
	50	24	14.0	3.52	13.0	3.02	5.8
negative control Beta-Oxynaphthoe- säure (BONS)	0	48	0.4	0.84	0.0	0.00	0.0
	2000	48	1.8	2.58	0.2	0.64	0.0

Chromosome aberrations in male and female Chinese hamsters

Dose: 0 mg/kg bodyweight (negative controls)

**Killing time: 12 h after administration (50 metaphases were analysed)**

[illegible]

**Table 3:**  
**Chromosome aberrations in male and female Chinese hamsters**

[illegible]

**Table 4:**  
**Chromosome aberrations in male and female Chinese hamsters**

[illegible]

Table 5:

Chromosome aberrations in male and female Chinese hamsters

Test substance: Beta-Oxynaphthoesäure (BONS)

Dose: 2000 mg/kg bodyweight

Killing time: 24 h after administration (50 metaphases were analysed)

Sex	Animal No.	No. of phases with aberrations incl. Gaps	No. of aberrations incl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
male	31	1	0	0	1	0	0	1								6.5
	32	1	0	0	1	0	0	1								6.1
	33	0	0	0	0	0	0									6.4
	34	0	0	0	0	0	0									7.1
	35	1	0	0	2	0	0	1	1							7.4
female	36	0	0	0	0	0	0									10.1
	37	0	0	0	0	0	0									12.4
	38	0	0	0	0	0	0									7.9
	39	1	0	0	1	0	0	1								11.7
	40	0	0	0	0	0	0									10.6
Mean		0.4	0.0	0.0	0.5	0.0	0.3	0.2								8.6
SD		0.52	0.0	0.0	0.71	0.0	0.48	0.42								2.36

Table 6:

## Chromosome aberrations in male and female Chinese hamsters

Test substance: Endoxan<sup>a</sup>

Dose: 50 mg/kg bodyweight (positive controls)

Killing time: 24 h after administration (50 metaphases were analysed)

Sex	Animal No.	No. of phases with aberrations incl. Gaps	No. of aberrations incl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
male	41	7	7	10	10							2	6		2 m	9.0
	42	8	6	20	17		2		3			1	9	1	1 di	6.8
	43	5	5	15	15		1	2				4	6		2 m	4.0
	44	6	5	11	9							2	6		1 m	8.8
	45	6	6	7	7		3						1	1	2 m	8.0
female	46	7	7	16	16							4	9	3		7.0
	47	11	10	14	12		1	1				8	3			10.0
	48	5	5	8	8				1	1		3	1		1m, 1ri	3.6
	49	8	7	12	10							4	5	1		8.0
	50	7	7	12	12							3	6	2	1 m	3.9
Mean		7.0*	6.5*	12.5*	11.6*	0.8	0.1	0.7	0.2	0.4	0.1	3.1	5.2	0.8	1.1	6.9
SD		1.76	1.51	3.89	3.44	1.14	0.32	1.06	0.63	0.97	0.32	2.18	2.82	1.03	0.88	2.32

\* =  $p \leq 0.01$

Table 7:

Chromosome aberrations in male and female Chinese hamsters  
 Test substance: Beta-Oxynaphthoesäure (BONS)  
 Dose: 0 mg/kg bodyweight (negative controls)  
 Killing time: 48 h after administration (50 metaphases were analysed)

Sex	Animal No.	No. of phases with aberrations incl. Gaps	No. of aberrations incl. Gaps	g	ig	b	ib	f	if	d	id	ma	ex	cd	others	MI %
male	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.4
	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.0
	54	1	0	0	0	0	0	0	0	0	1	0	0	0	0	9.7
	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.2
female	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.1
	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.6
	58	1	0	0	0	0	0	0	0	0	1	0	0	0	0	8.6
	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
Mean		0.2	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.2						8.8
SD		0.42	0.0	0.0	0.42	0.0	0.0	0.42	0.0	0.42						1.75

Chromosome aberrations in male and female Chinese hamsters  
Test substance: Beta-Oxynaphthoesäure (BONS)  
Dose: 2000 mg/kg bodyweight  
Killing time: 48 h after administration (50 metaphases were analysed)

[illegible]



30NS

Study of the mutagenic potential of the compound

2-Hydroxynaphtalin-3-carbonsäure

in strains of *Salmonella typhimurium* (Ames Test)  
and *Escherichia coli*

Report No. 370/82

This report contains the unpublished research findings of Hoechst scientists. It should not be published in whole or in part, or referred to in any publication without authorisation from the company.

Date: June 16, 1982

Responsibilities:

Dr. Jung  
Dr. Weigand

Department of Toxicology  
Industrial Toxicology  
Hoechst AG Frankfurt/Main

Contents

	Page
1. Summary	3
2. Introduction	4
3. Material and Methods	4 - 6
4. Results	6 - 7
5. Tables	8 - 17
6. Reference	18

## 1. Summary

2-Hydroxynaphtalin-3-carbonsäure was tested for mutagenicity with the strains TA 100, TA 1535, TA 1537, TA 1538, TA 98 of Salmonella typhimurium and Escherichia coli WP2uvrA.

The mutagenicity studies were conducted in the absence and in the presence of a metabolizing system derived from rat liver homogenate. A dose range of 5 different doses from 4 µg/plate to 1 000 µg/plate was used.

Control plates without mutagen showed that the number of spontaneous revertant colonies was similar to that described in the literature. All the positive control compounds gave the expected increase in the number of revertant colonies.

Toxicity: The test compound proved to be toxic to the bacteria at 500 or 1 000 µg/ plate. 1 000 µg/plate was chosen as top dose level for the mutagenicity study.

Mutagenicity: In the absence of the metabolic activation system the test compound did not show a dose dependent influence in the number of revertants in any of the bacterial strains due to mutagenicity. Also in the presence of metabolic activation system, treatment of the cells with 2-Hydroxynaphtalin-3-carbonsäure did not result in relevant increases in the number of revertant colonies.

Summarizing, it can be stated that 2-Hydroxynaphtalin-3-carbonsäure is not mutagenic in these bacterial test systems neither with nor without exogenous metabolic activation at the dose levels investigated.

## 2. Introduction

This report describes experiments performed in a short term test using the procedure of the Salmonella / mammalian-microsome-mutagenicity test (Ames Test) (1,2) to assess the mutagenic potential of the test material in amino acid-dependent strains of Salmonella typhimurium and a strain of Escherichia coli described by Green (3). By the use of liver homogenate the test takes into account the mammalian metabolism of the compound to be tested. The requirement for metabolic activation was investigated by incorporating into the test an activation system by nicotinamide-adenine dinucleotide phosphate (NADP<sup>+</sup>) -cytochrome P<sub>450</sub> dependent mixed function oxidase enzymes of the liver. The 9 000 g supernatant of rat liver homogenate has been shown to be very useful in metabolic activation of foreign compounds. The animals were pretreated with Aroclor 1254 as an inducer of several drug metabolizing enzymes (4).

In the Ames test with Salmonella typhimurium strains the effect of the test compound upon the number of back mutations to histidine prototrophy using histidine auxotrophic mutants is investigated. Using Escherichia coli WP2uvrA, a tryptophan dependant auxotroph strain, mutagenicity is based on reversion to tryptophan independence. The strains TA 100 und TA 1535 were originally derived by a substitution mutation, the strains TA 1537, TA 1538 and TA 98 by frame shift mutations from histidine prototrophic bacteria. All five Salmonella strains are deficient in the complete structure of their lipopolysaccharide layer and in DNA excision repair system (2). TA 98 and TA 100 possess a modified postreplication DNA repair system which frequently causes an increase in the rate of mutations (5). Strain WP2 carries a defect in one of the genes for tryptophan biosynthesis and is deficient in the uvrA system of DNA repair. The reversion can be induced by a base change (substitution).

## 3. Material and Methods

2-Hydroxynaphtalin-3-carbonsäure has been received as a yellow crystalline powder. It was stored in the dark at room temperature. At the day of the experiment the test substance was dissolved in DMSO at appropriate concentrations.

### Preparation and storage of liver homogenate fraction ("S-9")

Male Sprague Dawley rats (200 - 300 g) receive a single intraperitoneal injection of Aroclor 1254 (500 mg/kg body weight) 5 days before sacrifice. Preparation is performed at 0 to 4°C using cold sterile solutions and glassware. The livers from at least 5 - 6 animals are removed and pooled, washed in 150 mM KCl (approximately 1 ml/g wet livers). The washed livers are cut into small pieces and homogenised in three volumes of KCl. The homogenate is centrifuged at 9 000 g for 10 minutes. The supernatant is the S-9 fraction. It is divided into small portions, rapidly frozen and stored at -80°C for not longer than 3 months.

### Preparation of S-9 Mix and concentration of cofactors

Sufficient S-9 fraction is thawed immediately before each test at room temperature. One volume of the S-9 fraction are mixed with 9 volumes of the S-9 cofactor solution and kept on ice until used. This preparation is termed S-9 Mix. The concentrations of the different compounds in the S-9 Mix are:

8 mM MgCl  
33 mM KCl  
5 mM glucose-6-phosphate  
4 mM NADP<sup>+</sup>  
100 mM phosphate buffer pH 7,4

### Bacteria

Bacteria are grown overnight in nutrient broth (25 g Oxid Nutrient Broth No 2/ liter) at 37°C. The suitable amount of bacteria in the cell suspension is checked by nephelometry. For inoculation, stock cultures which are stored at - 80°C, are used. The compound is tested with the strains Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and E. coli WP2uvrA. Identification of the different bacterial strains is performed periodically as described (2,3).

### Mutagenicity experiments

Top agar is prepared for the Salmonella strains by mixing 100 ml agar (0,6 % agar, 0,5 % NaCl) with 5 ml of a 1,0 mM histidine and 5 ml of 1,0 mM biotin solution. With E. coli histidine is replaced by tryptophan (5 ml, 0,5 mM). The following ingredients are added (in order) to 2 ml of molten top agar at 45°C:

0,1 ml test compound solution  
0,1 ml of an overnight nutrient broth culture of the bacterial tester strain  
0,5 ml S-9 Mix (if required) or buffer

After mixing, the liquid is poured into a petridish with minimal agar (1,5 % agar, Vogel-Bonner E medium with 2 % glucose). After incubation for 48 to 72 hours at 37°C in the dark, colonies (his<sup>+</sup> revertants) are counted.

#### Positive controls

Positive control plates were included for each strain. The following substances were used as positive controls.

a) without metabolic activation:

Na-azide: TA 100, TA 1535;  
9-Aminoacridine: TA 1537;  
2-Nitrofluorene: TA 98, TA 1538  
N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG): WP2uvrA

b) with metabolic activation

Benzo[a]pyrene: TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2uvrA  
2-Aminoanthracene: TA 98, TA 100, TA 1535, TA 1537, TA 1538, WP2uvrA

#### Toxicity experiments and dose range finding

Preliminary toxicity tests were performed with all tester strains using a small number of plates to calculate an appropriate dose range. A reduced rate of spontaneously occurring colonies as well as visible thinning of the bacterial lawn were used as indicator for toxicity. Thinning of the bacterial lawn was controlled microscopically.

In combination with the main experiment, toxicity testing was performed as follows: 0,1 ml of the different dilutions of the test compound were thoroughly mixed with 0,1 ml of  $10^{-6}$  dilution of the overnight culture of TA 100 and plated with histidine and biotin rich top agar (3 plates per dose). The solvent control is compared with the number of colonies per plate in the presence of the test compound. Results are given as a ratio of these values. (= surviving fraction).

#### 4. Results

2-Hydroxynaphtalin-3-carbonsäure was tested for mutagenicity with Salmonella typhimurium strains TA 100, TA 1535, TA 1537, TA 1538, TA 98 and E. coli WP2uvrA with and without the addition of a metabolic activation system. The results obtained with the test material and positive control compounds are presented in table 1 to 9. The number of colonies per plate with each strain as well as mean values of 3 plates, corrected to the next whole number are given.

##### I. Sterility checks and control plates

Sterility of S-9 mix and the test compound was indicated by the absence of contamination on test material and S-9 mix sterility check plates. Control plates (background control and positive controls) gave the expected number of colonies.

## II. Toxicity test:

2-Hydroxynaphtalin-3-carbonsäure was tested at doses of 4 to 10 000 µg/plate (table 1) and proved to be toxic at doses of 500 or 2 500 µg/plate. Thinning of the bacterial lawn and a reduction in the number of colonies have been observed at these doses. These are conditions where the test compound can be tested with limited sensitivity.

For mutagenicity testing 1 000 µg/plate was chosen as the highest dose.

## III. Mutagenicity test with 2-Hydroxynaphtalin-3-carbonsäure

The test compound did not cause a significant increase in the number of colonies with any of the tester strains either in the absence or presence of S-9 mix (table 2 - 7, 9).

However a small increase in the number of colonies was obtained with TA 1537 in the absence of metabolic activation system. Therefore the test was repeated with TA 1537 in a second independent experiment.

No dose dependent effect was obtained (table 9).

Thus the effect of the first experiment can be explained by the very low spontaneous rate of revertant colonies with the control plates.

It is concluded that the test substance is not mutagenic in these bacterial test systems neither in the absence nor in the presence of an exogenous metabolizing system.

This test was performed according to the methods described. No unforeseen circumstances were observed which have affected the quality and integrity of this study.

Dr. Jg/Bo  
June 16, 1982

### Responsibilities:

Department of Toxicology  
- Industrial Toxicology -  
HOECHST AKTIENGESELLSCHAFT

*R. Jung*

Dr. Jung  
Study director

*E. Weigand*

Dr. Weigand  
Industrial Toxicology

Table 1: Toxicity experiment and dose range finding on 2-Hydroxynaphthalin-3-carbonsäure

Number of revertant colonies obtained with *Salmonella typhimurium*  
strain TA 1535, TA 1537, TA 1538, TA 100, TA 100, TA 98, and *E. coli* WP2uvrA

Dose (µg/plate)	Metabolic activation	Strain of <i>Salmonella typhimurium</i>					<i>Escherichia coli</i>	
		TA 100	TA 1535	TA 1537	TA 1538	TA 98	WP2uvrA	
0 (DMSO)	-	137	14	4	15	31	22	
4	-	139	15	9	14	25	22	
20	-	143	19	11	16	23	17	
100	-	158	22	8	8	18	27	
500	-	71*	13*	15*	14*	11*	21	
2 500	-	6*	6*	**	3**	**	7*	
10 000	-	0**	0**	0**	0**	0**	5*	
0 (DMSO)	+	107	6	9	7	32	23	
4	+	164	12	10	14	39	26	
20	+	81	11	3	13	22	30	
100	+	134	14	8	11	25	21	
500	+	141*	6	12	10*	20*	16	
2 500	+	24*	3**	6*	5*	15*	9*	
10 000	+	0**	0**	14*	0**	0**	0**	

\* : incomplete bacterial lawn

\*\* : no bacterial lawn

- : absence

+ : presence

Table 2: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

TA 100

Number of revertant colonies per plate and mean values  
using Salmonella typhimurium strain TA 100

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate	Surviving fraction
0 (DMSO)	-	153	140, 171, 149	1,0
4	-	157	167, 153, 151	1,0
20	-	170	160, 188, 163	1,0
100	-	146	158, 143, 138	0,8
500	-	124	101, 134, 137	0,4
1 000	-	50	42, 60, 48,*	0
0 (DMSO)	+	170	180, 156, 174	1,0
4	+	161	175, 167, 140	1,2
20	+	160	172, 175, 134	1,0
100	+	146	153, 159, 126	1,0
500	+	136	137, 130, 141	0,7
1 000	+	68	64, 80, 59,*	0,01

- : absence

+ : presence

\* : incomplete bacterial lawn

Table 3: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

TA 1535

Number of revertant colonies per plate and mean values  
 using Salmonella typhimurium strain TA 1535

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	-	11	13, 7, 12
4	-	18	18, 18, 18
20	-	14	16, 11, 15
100	-	14	17, 12, 13
500	-	13	18, 14, 8
1 000	-	8	5, 6, 13,*
0 (DMSO)	+	11	6, 11, 16
4	+	14	16, 16, 10
20	+	8	12, 6, 6
100	+	13	13, 14, 12
500	+	11	10, 14, 10
1 000	+	9	9, 8, 10,*

- : absence

+ : presence

\* : incomplete bacterial lawn

Table 4: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

TA 1537

Number of revertant colonies per plate and mean values  
using Salmonella typhimurium strain TA 1537

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	-	3	2, 2, 4
4	-	6	10, 5, 3
20	-	7	7, 6, 9
100	-	6	4, 9, 6
500	-	10	9, 11, 10,*
1 000	-	11	10, 4, 18,*
0 (DMSO)	+	9	6, 8, 12
4	+	13	14, 10, 15
20	+	5	6, 2, 6
100	+	11	10, 13, 10
500	+	11	7, 13, 13
1 000	+	7	7, 10, 3,*

- : absence

+ : presence

\* : incomplete bacterial lawn

Table 5: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

TA 1538

Number of revertant colonies per plate and mean values  
 using Salmonella typhimurium strain TA 1538

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	-	10	12, 6, 11
4	-	10	7, 7, 17
20	-	11	13, 11, 9
100	-	6	5, 5, 7
500	-	9	10, 7, 11
1 000	-	6	7, 6, 4,*
0 (DMSO)	+	12	14, 12, 9
4	+	11	21, 5, 7
20	+	10	9, 14, 7
100	+	13	9, 13, 16
500	+	13	11, 17, 10
1 000	+	12	15, 7, 13,*

- : absence

+ : presence

\* : incomplete bacterial lawn

Table 6: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

TA 98

Number of revertant colonies per plate and mean values  
 using *Salmonella typhimurium* strain TA 98

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	-	18	22, 17, 15
4	-	22	25, 20, 21
20	-	18	15, 18, 20
100	-	18	22, 13, 18
500	-	17	16, 17, 17
1 000	-	19	22, 22, 13,*
0 (DMSO)	+	24	19, 36, 18
4	+	28	23, 30, 30
20	+	28	24, 35, 25
100	+	23	22, 27, 19
500	+	26	21, 33, 23
1 000	+	16	17, 15, 16,*

- : absence

+ : presence

\* : incomplete bacterial lawn

Table 7: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

WP2uvrA

Number of revertant colonies per plate and mean values  
 using Escherichia coli strain WP2uvrA

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	-	26	22, 34, 23
4	-	25	23, 25, 26
20	-	25	18, 30, 28
100	-	27	33, 21, 26
500	-	30	33, 34, 23
1 000	-	6	8, 7, 2,*
0 (DMSO)	+	39	36, 44, 38
4	+	41	38, 40, 45
20	+	43	38, 48, 42
100	+	40	37, 42, 40
500	+	37	45, 28, 37
1 000	+	8	6, 7, 10,*

- : absence

+ : presence

\* : incomplete bacterial lawn

Table 8: mutability (positive controls) and sterility test of the experiment with 2-Hydroxynaphtalin-3-carbonsäure

Number of revertant colonies obtained and mean values  
 using Salmonella typhimurium and Escherichia coli strains

Strain	Compound	Dose (µg/plate)	Metab. activation	Mean value	Colonies per plate
TA 100	Sodium azide	1	-	639	639, 630, 649
TA 1535	Sodium azide	1	-	445	409, 467, 460
TA 1537	9-Amino- acridine	50	-	219	247, 218, 191
TA 1537	9-Amino- acridine (second experiment)	50	-	526	641, 357, 580
TA 1538	2-Nitro- fluorene	5	-	820	850, 990, 619
TA 98	2-Nitro- fluorene	5	-	641	609, 645, 669
WP2uvrA	MNNG	5	-	734	772, 764, 667
-	2-Hydroxy- naphtalin-3-carbonsäure	1 000 µg	-	0	0, 0, 0

Table 8a: mutability (positive controls) and sterility test of the experiment with 2-Hydroxynaphtalin-3-carbonsäure

Number of revertant colonies obtained and mean values using Salmonella typhimurium and Escherichia coli strains

Strain	Compound	Dose (µg/plate)	Metab. activation	Mean values	Colonies per plate
TA 100	Aminoanthracene	1	+	1071	710, 1720, 783
TA 1535	Aminoanthracene	1	+	162	174, 145, 166
TA 1537	Aminoanthracene	1	+	80	83, 75, 82
TA 1538	Aminoanthracene	1	+	1116	1126, 1106, 1117
TA 98	Aminoanthracene	1	+	787	1071, 364, 926
WP2uvrA	Aminoanthracene	10	+	252	251, 276, 230
TA 100	Benzo[a]pyrene	10	+	582	558, 706, 483
TA 1535	Benzo[a]pyrene	10	+	23	21, 27, 20
TA 1537	Benzo[a]pyrene	10	+	176	165, 189, 173
TA 1538	Benzo[a]pyrene	10	+	277	270, 272, 290
TA 98	Benzo[a]pyrene	10	+	639	484, 623, 509
WP2uvrA	Benzo[a]pyrene	10	+	82	71, 74, 102
-	S-9 mix	500 µl	+		0, 0, 0
-	2-Hydroxynaphtalin-3-carbonsäure	1 000 µg	+		0, 0, 0

Second experiment:

Table 9: Mutagenicity experiment with 2-Hydroxynaphtalin-3-carbonsäure with and without metabolic activation

TA 1537

Number of revertant colonies per plate and mean values  
 using Salmonella typhimurium strain TA 1537

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	-	6	4, 8, 7
4	-	4	3, 2, 6
20	-	3	2, 3, 4
100	-	5	3, 5, 6
500	-	7	8, 7, 7
1 000	-	5	**, 4, 6, *

- : absence
- + : presence
- \* : incomplete bacterial lawn
- \*\* : no bacterial lawn

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u-k181

*beta-hydroxynaphthoic acid (CAS 92-70-6)*

**BONS TTR**  
**BONS TRTR**  
other Registry Number 12235-60-8, 12235-61-9

**CAS 92-70-6**  
**Prod.-Nr.: GSVB 155/255**  
**OF**

Lit.Rech. am 04.03.1987 in RTECS, TDB und Toxline ohne brauchbare Ergebnisse

**BONS TTR**

Bericht Dr. Rupprich, Dr. Wg. vom 02.12.1983, HOE 83.0661

Akute orale Toxizität LD 50 (Ratte m. u. w.): 823 mg/kg KG

Bericht Dr. Rupprich, Dr. Holl vom 29.09.1983, HOE 83.0508

Haut (Kaninchen): nicht hautreizend

Bericht Dr. Rupprich, Dr. Wg. vom 08.10.1983, HOE 83.0515

Schleimhaut (Kaninchenauge):  
reizend am Auge  
Gefahr ernster Augenschäden  
R 41

Bericht Dr. Jung, Dr. Wg. vom 18.06.1982, HOE 370/82

Nicht mutagen mit oder ohne metabolische Aktivierung.

**BONS TRTR**

Bericht Dr. Rupprich, Dr. Wg. vom 05.01.1984, HOE 83.0665

Aktue orale Toxizität LD 50 (Ratte m. u. w.): 1.040 mg/kg KG

Bericht Pharma Forschung Toxikologie, Th. Hofmann, R. Jung vom 02.04.1987

Neubewertung der Haut- und Schleimhautverträglichkeit nach OECD:

Auge (Kaninchen):  
Haut (Kaninchen):  
reizend und R 41 (Gefahr ernster Augenschäden)  
nicht reizend



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

Susan P. Engelman, Vice President  
Environmental Health & Safety Affairs  
Hoechst Celanese Corporation  
Route 202-206  
P.O. Box 2500  
Somerville, New Jersey 08876-1258

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MAR 15 1995

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All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)  
Attn: TSCA Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

*Terry R. O'Bryan*

Terry R. O'Bryan  
Risk Analysis Branch

Enclosure

12976A



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## EPA INFORMATION REQUESTS

Document ID: 8EHQ-94-12976  
8EHQ-1094-13218

### EPA requests:

1. ☐ No additional information at this time.
2. ☐ Additional information or clarification on
3. ☐ A full copy of the final report (including the actual experimental protocol, applicable results of gross or histopathologic examinations, data, results of any statistical analyses, etc.) from each study mentioned in your submission.
4. ☒ A description of all voluntary actions taken by your company in response to the findings indicated in your submission.
5. ☐ A complete copy of the current and/or revised Material Safety Data Sheets and labels for the following chemical(s) listed in your submission:  

_____	_____
_____	_____
_____	_____
6. ☐

Please direct questions regarding these requests to Mr. Terry O'Bryan (202-260-3483) or Mr. John Myers (202-260-3543) of the OPPT Risk Analysis Branch.

### Triage of 8(e) Submissions

Date sent to triage: APR 22 1995

NON-CAP

CAP

Submission number: 12976A

TSCA Inventory:   

N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

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GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): \_\_\_\_\_

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

#### For Contractor Use Only

entire document:   

1 2

pages

1, 2

pages

1, 2, PAGES

Notes:

Contractor reviewer:   

POR

Date:   

12/3/94

CECATS TRIAGE TRACKING DATABASE ENTRY FORM

CECATS DATA: Submission # BEHQ-0994-12976 SEQ. A

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Hoechst Celanese Corporation

INFORMATION REQUESTED: FLWP DATE: 0501 NO INFO REQUESTED  
 0502 INFO REQUESTED (TECH)  
 0503 INFO REQUESTED (VOL ACTIONS)  
 0504 INFO REQUESTED (REPORTING RATIONALE)  
 DISPOSITION:  
 0600 REFER TO CHEMICAL SCREENING  
 0606 CAP NOTICE

0601 NO ACTION REPORTED  
 0602 STUDIES PLANNED WITHIN 6 MONTHS  
 0603 NOTIFICATION OF WORKING WITHIN 6 MONTHS  
 0604 LABORATORY TESTS  
 0605 PROCESSING WITHIN 6 MONTHS  
 0606 APPAUSE DISCONTINUED  
 0607 PRODUCTION DISCONTINUED  
 0608 CONFIDENTIAL

SUB. DATE: 08/24/94 CSRAD DATE: 11/09/94

CHEMICAL NAME: [REDACTED] CAS# 92-70-6

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEMPHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECOAQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCURRENCE/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAMAGE/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0248 PRODUCE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PRODCOMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0259 OTHER	01 02 04
0211 CHIR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

PRODUCTION:

USE:

TOXICOLOGICAL CONCERN:

SPECIES

ONGOING REVIEW

TRIAGE DATA NON-CBI INVENTORY

In Vitro  
 Ham  
 LOW  
 MED  
 HIGH

YES (DROP/REFER)  
 NO (CONTINUE)  
 REFER

CAS SR  
 YES  
 NO

IN INVENTORY

12-200213 10/1/94 (W)

5) 8EHQ-0994-12976: Rank - medium.

Chemical:  $\beta$ -hydroxynaphthoic acid (CAS# 92-70-6).

Study of the mutagenic potential of the compound 2-Hydroxynaphthalin-3-carbonsaure in strains of Salmonella typhimurium (Ames Test) and Escherichia coli, Hoechst Aktiengesellschaft, Frankfurt am Main, dated June 16, 1982: Negative for gene mutations in Salmonella typhimurium in strains TA98, TA100, TA1535, TA1537 and TA1538 both without and with metabolic activation.

Negative for gene mutations in Escherichia coli strain WP2uvrA both without and with metabolic activation.

Chromosome Aberrations In Vitro in V79 Chinese hamster Cells, Pharma Research Toxicology and Pharmacology, Frankfurt am Main, Germany, dated January 18, 1989: Positive for chromosome mutations in the form of chromosome aberrations without but not with metabolic activation in Chinese hamster V79 cells in vitro.

Chromosome Aberrations In Vivo Cytogenetic Test in Bone Marrow Cells of the Chinese Hamster, Hoechst Aktiengesellschaft, Frankfurt am Main, dated October 19, 1993: Negative for chromosome mutations (aberrations) in the bone marrow of Chinese hamsters exposed in vivo by oral gavage.